#12

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Patent of

Yukio SYUKUDA et al.

Patent No. 4,455,297 Issue Date: June 19, 1984

Serial No. 408,563 Filed: August 16, 1982

For: METHOD FOR PRODUCING PERTUSSIS TOXOID

LETTER ACCOMPANYING APPLICATION FOR EXTENSION

BOX PAT. EXT.
Honorable Commissioner of
Patents and Trademarks
Washington, DC 20231

Sir:

Enclosed herewith is an APPLICATION FOR EXTENSION for filing as of this date; kindly also make of record the following:

FEES FOR AMENDED CLAIMS

Excess independent claims at $72 each - ___	$
Excess total claims at $20 each -	$
First multiple dependent claim at $220 extra -	$

EXTENSION OF TIME PETITION

If this paper is filed outside the regular shortened period for response, applicant(s) petition(s) for the minimum extension of time needed to effect timely filing of the instant paper, calculated as being for a total of _____ month(s), and the fee being	$
EXTENSION OF TERM OF PATENT FEE	$1,000.00
[X] TOTAL FEE: Our check is included for:	$1,000.00

[X] Applicant(s) generally authorize(s) payment of any required fee for the filing of this paper (even if different from any calculation above) to our Deposit Account 23-0783 under our general authorization under 37 CFR 1.17.

WEGNER, CANTOR, MUELLER & PLAYER
P. O. BOX 18218
WASHINGTON, DC 20036-8218
(202) 887-0400
Atty. Doc.: 8700-5512
DATE: February 14, 1992
DPM:ldc/2.53

Respectfully submitted,

Douglas P. Mueller
Reg. No. 30,300

100 DH 02/19/92 4455297 2 111 1,000.00 CK

111/1000.00

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

Yukio SYUKUDA et al.

Patent No. 4,455,297 Issue Date: June 19, 1984

Serial No. 408,563 Filed: August 16, 1982

For: METHOD FOR PRODUCING PERTUSSIS TOXOID

APPLICATION FOR EXTENSION OF TERM FOR U.S. PATENT NO. 4,455,297

BOX PAT. EXT.
Honorable Commissioner of
Patents and Trademarks
Washington, DC 20231

Sir:

Takeda Chemical Industries, Ltd., owner of U.S. Patent No. 4,455,297 through Assignment recorded at Reel 3912, Frame 502, in parent application 229,931 filed January 30, 1981, hereby requests extension of the term of U.S. Patent No. 4,455,297. A check in the amount of $1,000.00 is filed herewith. Any deficiency can be charged to Deposit Account No. 23-0783.

The following information is provided in support of the application for extension. The information is arranged in accordance with 37 CFR 1.740.

1. IDENTIFICATION OF THE APPROVED PRODUCT

The approved product is a vaccine for diphtheria, tetanus and pertussis (whooping cough). The product will be marketed under the brand name "ACEL-IMUNE". The present application concerns a patent directed to the pertussis component of the three-component vaccine.

A copy of the approved labelling for this product is attached as Exhibit 1.

The pertussis toxoid of "ACEL-IMUNE" is prepared by a process which includes a step of innoculating Tohama phase I strain of Bordetella pertussis to form a seed culture. The seed culture is added to an appropriate production medium and incubated. The culture's supernatant is concentrated by salting out with the use of ammonium sulphate.

The concentrate is then dialyzed and subjected to sucrose density gradient centrifugation to remove endotoxin. A 1 to 26% (w/w) sucrose gradient is used. The centrifuge rotor is driven at R max of about 67,000-90,000 G for about 17 to 19 hours. Fractions which are high in HA-reactivity and low in endotoxin are collected and pooled.

Following the removal of endotoxin, the fluid containing pertussis exotoxin is diluted with phosphate-buffered saline and subjected to flocculation treatment with formaldehyde, which is done by adding to the fluid formalin in the absence of basic amino acids. The formalin is added stepwise, yielding a final concentration of 0.4% (v/v). The mixture is incubated at about 39°C for about 5 to 10 days, which is sufficient to substantially detoxify the pertussis exotoxin.

After dialysis, the flocculent mass in the resulting suspension is dispersed through ultrasonication (25 KHz), followed by filtering to obtain the final pertussis toxoid fluid.

2. IDENTIFICATION OF THE STATUTE UNDER WHICH REVIEW OCCURRED

Regulatory review of ACEL-IMUNE occurred under §505 of the Federal Food, Drug, and Cosmetic Act.

3. IDENTIFICATION OF THE DATE OF PERMISSION

Permission for commercial marketing was granted under §505 of the Federal Food, Drug, and Cosmetic Act on December 17, 1991.

4. IDENTIFICATION OF ACTIVE INGREDIENTS

ACEL-IMUNE is a three-component vaccine, containing namely, a diphtheria toxoid, a tetanus toxoid and a pertussis toxoid. The diphtheria toxoid and tetanus toxoid have been previously approved as vaccines for diphtheria and tetanus respectively. These were approved on March 24, 1947 under the Public Health Service Act 58 Stat. 682. The marketing applicant was American Cyanamid Co. through Lederle Laboratories, the same marketing applicant as for Acel-Immune. The diphtheria and tetanus toxoids were not previously approved with the present pertussis toxoid of ACEL-IMUNE, and the pertussis toxoid of ACEL-IMUNE has not been previously approved for any commercial marketing or use.

5. SUBMISSION OF THIS EXTENSION APPLICATION

This extension application is being submitted within the 60-day period permitted for submission. The 60-day period expires on Saturday, February 15, 1992. The last day on which the application

could be submitted is therefore Tuesday, February 18, 1992 (Monday, February 17 being a Federal holiday).

6. IDENTIFICATION OF THE PATENT FOR WHICH EXTENSION IS BEING SOUGHT

Extension is sought for U.S. Patent No. 4,455,297. This patent issued on June 19, 1984 and currently will expire on June 19, 2001. The inventors for this patent are Yukio Syukuda, Hideo Watanabe and Shigeo Matsuyama.

7. PATENT COPY

A complete copy of U.S. Patent No. 4,455,297 is included as Exhibit 2.

8. COPIES OF ANY DISCLAIMERS, CERTIFICATE OF CORRECTION, RECEIPT OF MAINTENANCE FEE PAYMENT OR REEXAMINATION CERTIFICATE

Exhibit 3 includes copies of the two maintenance fee receipts for this patent. Also included in Exhibit 3 is a copy of a recently-filed Request for Certificate of Correction seeking correction of a minor error in claim 1 of the patent as printed.

9. APPLICATION OF THE PATENT CLAIMS TO THE APPROVED PRODUCT

Each of claims 1-5, 7 and 8 of U.S. Patent No. 4,455,297 covers the method of producing the pertussis toxoid of "ACEL-IMUNE". This is discussed in detail below, with reference to the previous description of the approved product.

Claim 1 of the patent is directed to a method of producing a pertussis toxoid, and includes the following steps:

1. Removal of endotoxin from a culture supernatant of a Bordetella pertussis phase I strain or a concentrate thereof.

2. Flocculating pertussis exotoxin in the resultant fluid by permitting formaldehyde to act upon the fluid in the substantial absence of basic amino acid.

3. Dispersing the flocculent mass by ultrasonication.

The pertussis toxoid of ACEL-IMUNE is produced by a method including each of these steps. Step 1 is met since a Tohama phase I strain of Bordetella pertussis is cultured, and endotoxin is removed. Step 2 is met since formaldehyde (through the addition of formalin) is allowed to act upon the resulting fluid, and basic amino acids are not present. Finally, the method of producing the pertussis toxoid of ACEL-IMUNE meets the third step of claim 1 since ultrasonication is used for dispersing the flocculent mass.

Claim 2 depends from claim 1, and further requires that flocculation is performed by admixing formalin or a dilution thereof with the fluid resulting from endotoxin removal in the absence of basic amino acid, followed by incubation. The process for producing the pertussis toxoid of ACEL-IMUNE utilizes a formalin solution for flocculation, without the presence of basic amino acids, and also incubates the mixture.

Claim 3 depends upon claim 2, and further requires that the incubation is continued until the pertussis exotoxin is substantially detoxified. The incubation of the process for

producing the pertussis toxoid of ACEL-IMUNE is sufficient to meet this requirement.

Claim 4 depends upon claim 2, and further requires that the amount of formalin be such as to give a concentration of about 0.1 to 0.6 v/v%, with the mixture being incubated at about 32-42°C for about 3-14 days. The process for producing ACEL-IMUNE falls within each of these ranges.

Claim 5 requires that the endotoxin removal of claim 1 is accomplished by centrifuging the cultured supernatant or concentrate thereof on a sucrose density gradient of about 0-60 w/w% at R max of about 62,000 to 122,000 G for about 10-24 hours. Again, the endotoxin removal step in the process of producing the ACEL-IMUNE pertussis toxoid falls within these ranges.

Claim 7 depends upon claim 1 and requires concentration of the supernatant by salting out with ammonium sulphate and removal of endotoxin from the resulting concentrate. The process for preparing the pertussis toxoid of ACEL-IMUNE does produce a concentrate by salting out with ammonium sulphate and removes endotoxin from the concentrate.

Claim 8 depends upon claim 1 and requires that the Bordetella pertussis phase I strain is the Tohama phase I strain. The process for producing the pertussis toxoid of ACEL-IMUNE utilizes the Tohama phase I strain.

10. INFORMATION FOR THE REGULATORY REVIEW

BB-IND 2417 was filed on June 24, 1986. Product license application 87-0406 was filed on September 1, 1987. PLA 87-0406 was granted on December 17, 1991.

11. ACTIVITIES UNDERTAKEN DURING THE REGULATORY REVIEW PERIOD

DATE	DESCRIPTION
	BB-IND 2417
06/24/86	Original IND (Parts I-XIV and XVI) clinical study 60-1-3
07/21/86	Part VI, Data Takeda 8135-2
07/24/86	Parts VIII-X, clinical study 60-1-4
08/04/86	Parts VIII-X, clinical study 60-1-2
08/04/86	Parts VIII-X, clinical study 60-1-1
08/21/86	Part X, IRB approval 60-1-4
08/26/86	Part IX, pre-invest. report
09/25/86	Parts VII-X, clinical study D60-P-11
10/16/86	Parts VIII-X, clinical study 60-2-1
10/16/86	Parts VIII-X, clinical study 60-2-2
10/29/86	Part IX, pre-invest. report
11/10/86	Parts VIII-X, clinical study 60-2-3
12/30/86	Part X, clinical study 60-1-4
04/15/87	Parts VI, VIII-X, clinical study 60-9-1
04/27/87	Part X, clinical study 60-9-1
04/29/87	Parts VII-X, clinical study 60-11-1
04/30/87	Part VII, labels 60-11
04/30/87	Part VII, labels 60-9
05/20/87	Parts VIII-X, clinical study 60-9-2
05/21/87	Parts VIII-X, clinical study 60-9-3
05/27/87	Parts VIII-X, clinical study 60-9-5
06/01/87	Parts VIII-X, clinical study 60-9-4

DATE	DESCRIPTION
06/10/87	Parts VIII-X, clinical study 60-9-6
06/19/87	Parts VIII-X, clinical study 60-9-7
06/19/87	Parts VIII-X, clinical study 60-9-8
06/19/87	Parts VIII-X, clinical study 60-9-9
06/26/87	Parts VII-X, clinical study 60-10-1
06/30/87	Parts VIII-X, clinical study 60-10-2
07/07/87	Parts VIII-X, clinical study 60-9-10
07/08/87	Parts VIII-X, clinical study 60-9-11
07/09/87	Parts VIII-X, clinical study 60-10-3
08/11/87	Parts VIII-X clinical study 60-9-8
08/24/87	Part X, clinical study 60-10
08/31/87	Part VIII, clinical study 60-9-9
09/01/87	PLA 87-0406
11/03/87	Parts VIII-X, clinical study 60-9-9
11/12/87	Part X, clinical study D60-P1-T2
11/16/87	Part X, clinical study, 60-11-1
12/02/87	Part VIII, clinical study D60-P1-T4
12/03/87	Part X, clinical study D60-P1-T4
12/14/87	Parts VIII-X, clinical study 60-10-6
12/28/87	Part X, clinical study D60-P2-T1
12/29/87	Parts VIII-X, clinical study 60-10-7
01/12/88	Parts VIII-X, clinical study 60-10-8
01/19/88	Part X, clinical study D60-P9-T9
01/21/88	Parts VIII-X, clincal study 60-10-9
02/23/88	Parts VIII-X, clinical study 60-10-10

DATE	DESCRIPTION
06/10/87	Parts VIII-X, clinical study 60-9-6
06/19/87	Parts VIII-X, clinical study 60-9-7
06/19/87	Parts VIII-X, clinical study 60-9-8
06/19/87	Parts VIII-X, clinical study 60-9-9
06/26/87	Parts VII-X, clinical study 60-10-1
06/30/87	Parts VIII-X, clinical study 60-10-2
07/07/87	Parts VIII-X, clinical study 60-9-10
07/08/87	Parts VIII-X, clinical study 60-9-11
07/09/87	Parts VIII-X, clinical study 60-10-3
08/11/87	Parts VIII-X, clinical study 60-9-8
08/24/87	Part X, clinical study 60-10
08/31/87	Part VIII, clinical study 60-9-9
09/01/87	PLA 87-0406
11/03/87	Parts VIII-X, clinical study 60-9-9
11/12/87	Part X, clinical study D60-P1-T2
11/16/87	Part X, clinical study, 60-11-1
12/02/87	Part VIII, clinical study D60-P1-T4
12/03/87	Part X, clinical study D60-P1-T4
12/14/87	Parts VIII-X, clinical study 60-10-6
12/28/87	Part X, clinical study D60-P2-T1
12/29/87	Parts VIII-X, clinical study 60-10-7
01/12/88	Parts VIII-X, clinical study 60-10-8
01/19/88	Part X, clinical study D60-P9-T9
01/21/88	Parts VIII-X, clinical study 60-10-9
02/23/88	Parts VIII-X, clinical study 60-10-10

DATE	DESCRIPTION
02/26/88	Part X, clinical study D60-P9-T1
03/02/88	Parts IX-X, clinical study 60-10-11
03/04/88	Part X, clinical study D60-P10-T4
03/07/88	Parts VIII-X, clinical study 60-10-12
03/08/88	Parts VIII-X, clinical study 60-10-13
03/10/88	Safety, clinical study 60-9-7
03/10/88	Safety, clinical study 60-9-8
03/17/88	Parts I, III, V-X, clinical study 60-12-1
03/31/88	Part X, clinical study D60-P9-T4
04/04/88	Parts VIII-X, clinical study 60-10-14
04/04/88	Part X, clinical study 60-12-1
04/11/88	Parts VI-X, clinical study 60-13-1
05/31/88	Parts VIII and IX, clinical study D60-P13-T1
06/17/88	Safety, death/Japan
06/24/88	Part X, clinical study 60-10-5
06/30/88	Response to CBER re: Submission 10/8/87
07/14/88	Letter to CBER re: Submission 11/3/87
07/19/88	Part X, clinical study D60-P9-T8
07/19/88	Part X, clinical study D60-P9-T6
07/19/88	Part X, clinical study D60-P10-T5
08/22/88	Part X, clinical study D60-P9-T7
08/22/88	Part X, clinical study D60-P9-T9
08/22/88	Part X, clinical study D60-P10-T7
08/23/88	Part X, clinical study D60-P9-T5
08/24/88	Part VIII, clinical study 60-9-2

DATE	DESCRIPTION
08/26/88	Part X, clinical study D60-P9-T11
09/01/88	Part X, clinical study D60-P9-T2
09/12/88	Response to CBER re: Submission 11/3/87
09/21/88	Part VII, clinical study 60-13-1
09/21/88	Parts VIII and X, clinical study D60-P9-T7
09/22/88	Safety, clinical study 60-10-14
10/07/88	Part X, clinical study 60-10-14
10/17/88	Part X, clinical study 60-10-11
10/17/88	Part X, clinical study 60-10-9
10/25/88	Letter to CBER re: Submission 3/7, 4/4, 4/11/88
10/27/88	Part X, clinical study 60-10-2
11/11/88	Safety, death/Japan
11/11/88	Part X, clinical study D60-P10-T9
11/11/88	Part X, clinical study D60-P2-T2
11/28/88	Safety, followup Submission 11/11/88, death/Japan
12/22/88	Response to CBER re: letter 10/25/88
01/09/89	Parts I-III and V, Led/Wyeth Procedures 7-1407-XX
01/16/89	Letter to CBEr re: Submission 11/11/88
01/19/89	Part X, clnical study 60-10-1
03/08/89	Parts VII and X, clinical study 60-10-1
03/13/89	Part X, clinical study 60-10-3
04/10/89	Part X, clinical study 60-10-9
04/13/89	Part X, clinical study 60-10-6
04/24/89	Parts VI-X, clinical study D60-P16-T3
04/16/89	Part X, clinical study D60-P9-T4

DATE	DESCRIPTION
04/28/89	Parts VIII-X, clinical study D60-P16-T1
05/05/89	Part X, clinical study D60-P9-T6
05/17/89	Part X, clinical study 60-10-11
05/17/89	Part X, clinical study 60-10-10
05/24/89	Ann. IRB approval
05/24/89	Amend. #3 60-10-7
06/12/89	Amend. #3 60-10-6
06/12/89	Ann. IRB approval
06/12/89	Parts VIII, IX, X - D60-P16-T2
06/16/89	Addl. clin. site
06/16/89	Annual Report
06/20/89	Ann. IRB approval
06/23/89	Amend. #3 60-10-5
07/05/89	Ann. IRB approval
07/19/89	60-18
07/24/89	60-17 (Parts VI, VII, VIII, IX, X)
07/25/89	60-10-13
07/27/89	60-18-2
08/01/89	Samples for DP 60-16
08/01/89	Addl. labeling for DP 60-10
08/08/89	Corrected pages for protocol (testing)
08/14/89	Addl. labeling for 60-10
08/14/89	Annual IRB approval - 60-10-2
08/14/89	IND Safety report 60-10-9
08/22/89	Amend #3 60-10-14

DATE	DESCRIPTION
08/31/89	Parts VIII, IX, X - 60-18-3 (B. Sullivan)
09/06/89	Part VIII - 60-16-1 (adds co-investigator)
09/07/89	Part VII - 60-16 addl. labeling
09/25/89	Part VIII - 60-16-4 addl. satellite clin. sites
10/02/89	Parts VIII, IX, X - 60-16-5 (Storokin)
10/02/89	Parts VIII, IX, X - 60-16-7 (Asmar)
10/03/89	Parts VIII, IX, X - 60-16-8 (Rothstein)
10/11/89	Parts VIII, IX, X - 60-16-6 (Black)
10/24/89	Part X - Amend. #3 D60-P16-T3
10/25/89	Addl. info for April 10 and May 10 submissions
10/27/89	Follow-up safety
10/30/89	Patient consent form and IRB approval D60-P16-T8
11/22/89	Addl. co-investigators (Part VIII)
12/21/89	Parts I, III, VI - Support of clin. study
01/12/90	Addl. info for July 24, 1989 submission
01/24/90	Clin. Invest., D60-P1
02/08/90	Parts VII, IX, X - 6012-1
02/27/90	Correspondence re meeting for 2/28/90
03/08/90	DP60-2
03/28/90	D60-P16-T1, Amendment #3
03/28/90	D60-P16-T2
03/28/90	D60-P16-T4, Amendment #3
04/04/90	Annual IRB approval
04/27/90	Testing results
05/18/90	Part VI, corrected testing results

DATE	DESCRIPTION
05/18/90	Request for more info. re letter of Dec. 21, 1989
06/08/90	Parts VII, VIII, IX, X - D60-P2-T1
06/11/90	Clinical study, D60-P16
06/15/90	Clinical study, D60-P9 (primary)
06/15/90	Clinical study, D60-P9 (18 mo. booster)
06/18/90	Clinical study, D60-P10
06/20/90	D60-P2-T3, Amendment #2
06/20/90	D60-P2-T2, Amendment #2
06/29/90	Completed clinical study
08/08/90	Annual IRB approval, 60-9-3, Sullivan
08/15/90	Annual IRB approval, 60-16-4, Plotkin
08/15/90	Annual IRB approval, 60-16-3, Reisinger
08/23/90	Annual IRB approval, 60-10-7, Mortimer
08/24/90	Annual IRB approval, 60-10-5, Sullivan
08/24/90	Annual IRB approval, 60-10-8, Gooch
08/24/90	Annual IRB approval, D60-P16-T1, Grossman
08/29/90	D60-P9-T2, transfer principal invest. from Nelson to Pomeranz
08/29/90	D60-P10-T11, transfer principal invest. from Nelson to Pomeranz
09/20/90	Annual IRB approval, 60-16-2, Glode
10/01/90	D60-P16-T4, sub-investigator, Chua
11/05/90	D60-P16-T7, Part X, Asmar
11/28/90	D60-P20, parts Vi, VII, VIII, IX, X - (Germany study)

DATE	DESCRIPTION
11/21/90	Part X, D60-P10-T5, annual IRB, Sullivan
12/21/90	Part X, D60-16-8, annual IRB, Rothstein
12/21/90	Part X, IRB approval of termination, D60-P18-T3, Sullivan
01/21/91	Clinical trial protocol for discussion at closed session, 01/29/91
02/01/91	Parts VII, VIII, X - D60-P19-T1, Glode
02/01/91	Parts VII, VIII, X - D60-P19-T2, Rothstein
02/18/91	Clinical study (also submitted to PLA 87-0406)
02/25/91	New investigator, Dr. Chua
04/04/91	Clinical study (Also submitted to PLA 87-0406)
04/12/91	Part X, 60-19-1, Amendment #1 - Glode
04/12/91	Part X, 60-19-2, Amendment #1 - Rothstein
05/21/91	Parts VII, X, 60-9-2, Pomeranz
05/24/91	Parts VI, VII, VIII, X - Initiation of German study
05/31/91	Parts VIII, X, 60-9, Starr
05/31/91	Parts VIII, X, 60-21 - 18 addl. investigators to German study
06/07/91	Part X, 60-9, Congeni
06/07/91	Part X, 60-9, Clin. Invest. Brochure
06/07/91	Parts VIII, X, 25 addl. investigators to German study
06/14/91	Parts VIII, X, 60-9, Gooch
06/14/91	Part X, 60-9, Sullivan

DATE	DESCRIPTION
06/17/91	Parts VIII, X - 16 addl. investigators to German study
06/18/91	Part X, 60-9, Mortimer
06/20/91	Answers to CBER Questions of 4/24/91
06/26/91	Part X, 60-9, Cherry
06/26/91	Parts VIII, X, 20 addl. investigators to German study
06/26/91	Parts VIII, X, 60-9, Townsend
06/26/91	Part X, 60-9, Prober
07/10/91	Part X, 60-9, Daum
07/11/91	Parts VIII, X - 6 additional investigators to German study
07/15/91	Parts VII, VIII, X - 12 additional investigators to German study
07/17/91	Safety Report
07/23/91	Parts VIII, X - 14 additional investigators to Germany study
07/29/91	Parts VIII, X - 2 additional invesitigators to German study
07/30/91	Correspondence re labeling of clinical vials for German study
07/30/91	Letter re supply of APDT for use in NIAID clinical trial
08/02/91	Parts VIII, X - 3 additional investigators to German study

DATE	DESCRIPTION
08/12/91	Parts VIII, X - 6 additional investigators to German study
08/13/91	Annual report for 1989, 1990, 1991
08/13/91	Part X - 60-9, Mortimer
08/16/91	Parts VIII, X - 3 additional investigators to German study
08/26/91	Parts VIII, X - 3 additional investigators to German study
09/04/91	Parts VIII, X - 1 additional investigator to German study
09/10/91	Parts VIII, X - 2 additional investigators to German study
09/16/91	Parts VIII, X - 1 additional investigator to German study
09/24/91	Follow-up information for safety report submitted on July 17, 1991
09/24/91	Parts VIII, X - 1 additional investigator to German study
10/01/91	Parts VIII, X - 2 additional investigators to German study
10/09/91	Parts VIII, X - 3 additional investigators to German study
10/22/91	Parts VIII, X - 12 additional investigators to German study

DATE	DESCRIPTION
10/29/91	Parts VIII, X - 7 dditional investigators to German study
11/05/91	Parts VI, VII, X - 60-10, Amendment #4
11/06/91	Part VII - 60-20 labels
11/06/91	Parts VIII, X - 15 additional investigators to German study
11/20/91	Parts VII, VIII, X - 9 additional investigators to German study
11/20/91	Letter re: supply of APDT for use in NIAID clinical trial
11/22/91	Parts VIII, X - 5 additional investigators to German study
11/26/91	Parts VIII, X - 60-10, Amendment #4
12/04/91	Part X - Amendment #4, D60-P10
12/09/91	Part X - Amendment #4, D60-P10
12/09/91	Parts VIII, X - 17 additional investigators to German study
12/13/91	Part X - Amendment #4, D60-P10
12/16/91	Parts VIII, X - 3 additional investigators to German study
12/17/91	Approval of PLA 87-0406

12. ELIGIBILITY FOR EXTENSION AND THE LENGTH OF EXTENSION CLAIMED

In the opinion of the applicant, U.S. Patent No. 4,455,297 is eligible for extension. It is believed that an extension of 1643 days, i.e., to December 17, 2005, is justified. The calculation of the extension is set forth below, it being noted that the marketing applicant acted with due diligence in pursuing both the IND and the PLA for ACEL-IMUNE.

The IND was pending from June 24, 1986 (after the issuance of U.S. Patent No. 4,455,297) through September 1, 1987, a total of 435 days. Reducing this total by one-half (after ignoring the half-day) leaves a total of 218 days. The PLA was pending from September 1, 1987 through December 17, 1991, including one leap year, a total of 1569 days. Adding the applicable IND period to the PLA period gives a total potential extension of 1787 days. This, when added to the original expiration date of June 19, 2001, would result in an expiration date of May 10, 2006.

Adding 14 years to the December 17, 1991 approval date gives a date of December 17, 2005. Since this is earlier than May 10, 2006, this date is selected. Since the patent issued before September 24, 1984 but the IND was not filed before September 24, 1984, the December 17, 2005 date is compared with the date obtained by adding 5 years to the original expiration date, i.e., June 19, 2006. Again, December 17, 2005 is earlier and this then is the extension which is available, which is calculated to be 1643 days (including one leap year). Therefore, an extension of 1643 days, to December 17, 2005, is requested.

13. DUTY OF DISCLOSURE

Applicant acknowledges the duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services or the Secretary of Agriculture any information which is material to the determination of entitlement to the extension sought.

14. FEE

A check for the required fee of $1,000 is filed herewith, with an appropriate Deposit Account authorization for any deficiency.

15. CORRESPONDENCE ADDRESS

Correspondence concerning this application should be directed to:

Douglas P. Mueller, Esq.
WEGNER, CANTOR, MUELLER & PLAYER
P. O. Box 18218
Washington, DC 20036-8218
(202) 887-0400
Atty. Doc. 8700-5512

16. DUPLICATE OF THE APPLICATION

A duplicate of this application, certified to be complete, is filed herewith.

17. OATH OR DECLARATION

The undersigned declares that:

1. He is an official of Takeda Chemical Industries, Ltd., owner by Assignment of U.S. Patent No. 4,455,297, authorized to obligate the corporation;

2. He has reviewed and understands the contents of the application for extension of U.S. Patent No. 4,455,297 attached hereto;

3. He believes U.S. Patent No. 4,455,297 is subject to extension pursuant to 37 CFR 1.710;

4. He believes an extension of the length claimed in section 12 above is justified under 35 USC 156 and the applicable regulations; and

5. He believes U.S. Patent No. 4,455,297 meets the conditions for extension of the term of a patent as set forth in 37 CFR 1.720.

6. He further declares under penalty of perjury of the laws of the United States that the foregoing is true to the best of his information and belief.

Feb. 10, 1992
DATE

Hiroshi Iwata
Mr. Hiroshi Iwata
General Manager

EXHIBIT 1

DOSAGE AND ADMINISTRATION

The dose is 0.5 mL to be given intramuscularly only.

A fourth and/or fifth dose with ACEL-IMUNE is indicated for children who have previously been immunized with at least three doses of whole-cell DTP vaccine.

The fourth dose consists of 0.5 mL of ACEL-IMUNE administered at approximately 18 months of age, and at least 6 months following the third DTP immunization.

A fifth dose consists of 0.5 mL of ACEL-IMUNE and is indicated at 4 to 6 years of age, preferably prior to entrance into kindergarten or elementary school. However, if the fourth dose of the basic immunizing series was administered after the fourth birthday, a booster prior to school entry is not considered necessary.[3]

Shake vigorously to obtain a uniform suspension prior to withdrawing each dose from the multiple dose vial. The vaccine should not be used if it cannot be resuspended.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit. (See **DESCRIPTION.**)

The vaccine should be injected intramuscularly. The preferred sites are the anterolateral aspect of the thigh or the deltoid muscle of the upper arm. The vaccine should not be injected in the gluteal area or areas where there may be a major nerve trunk. Before injection, the skin at the injection site should be cleansed and prepared with a suitable germicide.

After insertion of the needle, aspirate to help avoid inadvertent injection into a blood vessel.

If a contraindication to the pertussis vaccine component occurs, Diphtheria and Tetanus Toxoids Adsorbed for pediatric use (DT) should be substituted for each of the remaining doses.

For either primary or booster immunization against tetanus and diphtheria of individuals 7 years of age and older, the use of Tetanus and Diphtheria Toxoids Adsorbed for Adult Use (Td) is recommended.[3,18]

HOW SUPPLIED

NDC 0005-1950-31 5.0 mL vial

STORAGE

DO NOT FREEZE. STORE REFRIGERATED, AWAY FROM FREEZER COMPARTMENT, AT 2°C TO 8°C (36°F TO 46°F).

REFERENCES

1. Mueller JH, Miller PA. Production of diphtheria toxin of high potency (100 Lf) on a reproducible medium. *J Immunol* 1941;40:21-32.
2. Mueller JH, Miller PA. Factors influencing the production of tetanus toxin. *J Immunol* 1947; 56:143-147.
3. Pillemer L, Grossberg DB, Wichler RE. The immunochemistry of toxins and toxoids. II. The preparation of immunologic evaluation of purified tetanal toxoid. *J Immunol* 1946; 54:213-224.
4. Arai H, Sato Y. Separation and characterization of two distinct hemagglutinins contained in purified leukocytosis promoter factor from Bordetella pertussis. *Biochimica et Biophysica Acta* 1975;444:765-782.
5. Diphtheria, tetanus and pertussis. Recommendations for vaccine use and other preventive measures -- Recommendations of the Immunization Practices Advisory Committee (ACIP). *MMWR* 1991; Vol. 40, No. RR-10.
6. Iosen J. Immunization of adults against diphtheria and tetanus. *NEJM* 1954; 251:455-466.
7. *Fed. Reg.* Vol. 50, No. 240, Dec. 13, 1985.
8. Reported incidence of notifiable diseases in the United States. *MMWR* 1970; 19(53):44.
9. Pertussis Surveillance - United States, 1986-1988. *MMWR* 1990; 39(4):57-66.
10. Cowell JL, Oda M, Burstyn DG, et al. Prospective protective antigens and animal models for pertussis. In: Leive L and Schlessinger D, eds. *Microbiology - 1984*. Washington, DC: American Society for Microbiology 1984; 172-175.
11. Shahin RD, Brennan MJ, Li ZM, et al. Characterization of the protective capacity and immunogenicity of the 69K Da outer membrane protein of Bordetella pertussis. *J Exper Med* 1990; 171(1):63-73.
12. Novotny P, Kobisch M, Cownley K, et al. Evaluation of *Bordetella bronchiseptica* vaccines in specific-pathogen-free piglets with bacterial cell surface antigens in enzyme linked immunosorbent assay. *Infect Immun* 1985; 50:190-198.
13. Manclark CR, Cowell JL. Pertussis. In: Germanier R, ed. *Bacterial Vaccines*. Orlando, FL: Academic Press, Inc. 1984; 69-106.
14. Report of the Task Force on Pertussis and Pertussis Immunization - 1988. Pediatrics Vol. 81, No. 6, Part 2, 939-984.
15. Kimura M, Kuno-Sakai H. Developments in pertussis immunisation in Japan. *The Lancet* 1990; 30-32.
16. Noble GR, Bernier RH, Esser EC, et al. Acellular and whole-cell pertussis vaccines in Japan. *JAMA* 1987; 257:1351-1356.
17. Pertussis - United States, 1982 and 1983. *MMWR* 1984; 33(40):573-575.
18. Isomura S, Suzuki S, Sato Y. Clinical efficacy of the Japanese acellular pertussis vaccine after intrafamiliar exposure to pertussis patients. Proceedings of the Fourth International Symposium on Pertussis, Joint IABS/WHO Meeting, Geneva, Switzerland, 1984. Develop. Biol. Standard. Vol. 61, pp 531-537 (S. Karger, Basel, 1985).
19. Aoyama T, Murase Y, Gonda T, Iwata T. Type-specific efficacy of acellular pertussis vaccine. *AJDC* 1988; 142:40-42.
20. Kato T, Goshima T, Nakajima N, Kaku H, Arimoto Y, Hayashi F. Protection against pertussis by acellular pertussis vaccines (Takeda, Japan): Household contact studies in Kawasaki City, Japan. *Acta Paediatr Jpn* 1989; 31:698-791.
21. Mortimer EA, Kimura M, Cherry JD, et al. Protective efficacy of the Takeda Acellular Pertussis Vaccine combined with diphtheria and tetanus toxoids following household exposure of Japanese children. *AJDC* 1990; 144:899-904.
22. Blumberg DA, Mink CM, Cherry JD, et al. Comparison of acellular and whole-cell pertussis-component diphtheria-tetanus-pertussis vaccines in infants. *J Pediatr* 1991; 119:194-204.
23. Morgan CM, Blumberg DA, Cherry JD, et al. Comparison of acellular and whole-cell pertussis-component DTP vaccines. *AJDC* 1990;144:41-45.
24. Blumberg DA, Mink CM, Cherry JD, et al. Comparison of an acellular pertussis-component DTP vaccine with a whole-cell pertussis-component DTP vaccine in 17 to 24 month-old children, with measurement of 69-kilodalton outer membrane protein antibody. *AJDC*. In press.
25. Data on file, Lederle Laboratories.
26. American Academy of Pediatrics. *Report of the Committee on Infectious Diseases*. 22nd ed. Elk Grove Village, IL: American Academy of Pediatrics. 1991.
27. Pertussis immunization: family history of convulsions and use of antipyretics - supplementary ACIP statement. *MMWR* 1987; 36(18):281-282.
28. Recommendation of the ACIP: Immunization of children infected with Human T-Lymphotropic Virus Type III/Lymphadenopathy-associated virus. *MMWR* 1986; 35(38):595-606.
29. Immunization of children infected with Human Immunodeficiency Virus - Supplementary ACIP statement. *MMWR* 1988; 37(12):181-183.
30. National Childhood Vaccine Injury Act. Requirements for permanent vaccination records and for reporting of selected events after vaccination. *MMWR* 1988; 37(13):197-200.
31. Fawcett HA, Smith NP. Injection-site granuloma due to aluminum. *Arch Dermatol* 1984; 120:1318-1322.
32. Adverse events following immunization. *MMWR* 1985. 34(3):43-47.
33. Rutledge SL, Snead OC. Neurological complications of immunizations. *J Pediatr* 1986; 109:917-924.
34. Sedlenska GK. Unusual neurological complications following tetanus toxoid administration. *J Neurol* 1977; 215:299-302.
35. Blumstein GI, Kreithen H. Peripheral neuropathy following tetanus toxoid administration. *JAMA* 1966; 198:1030-1031.
36. Reinstein L, Pargament JM, Goodman JS. Peripheral neuropathy after multiple tetanus toxoid injections. *Arch Phys Med Rehabil* 1982; 63:333-334.
37. Isaacs P, Dyck PJ, Mulder DW. Natural history of brachial plexus neuropathy. *Arch Neurol* 1972; 27:109-117.
38. Quast U, Hennessen W, Whamark RH. Mono- and polyneuritis after tetanus vaccination. *Dev et Bio Stand* 1979; 43:25-32.
39. Holliday PL, Bauer RB. Polyradiculoneuritis secondary to immunization with tetanus and diphtheria toxoids. *Arch Neurol* 1983;40:56-57.
40. Fenichel GM. Neurological complications of tetanus toxoid. *Arch Neurol* 1983; 40:390.
41. Pollard JD, Selby G. Relapsing neuropathy due to tetanus toxoid. *J Neurol Sci* 1978; 37:113-125.
42. Newton N, Janah A. Guillain-Barre syndrome after vaccination with purified tetanus toxoid. *S Med J* 1987; 80:1053-1054.

Lederle

LEDERLE LABORATORIES DIVISION
American Cyanamid Company, Pearl River, NY 10965

REV. 12/91

11693-91
D1

DIPHTHERIA and TETANUS TOXOIDS and ACELLULAR PERTUSSIS VACCINE ADSORBED ACEL-IMUNE®

DESCRIPTION

Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed, ACEL-IMUNE®, is a sterile combination of PUROGENATED® Diphtheria Toxoid, PUROGENATED® Tetanus Toxoid, and Acellular Pertussis Vaccine, which is adsorbed to an aluminum salt. ACEL-IMUNE is for intramuscular use only. After shaking, the vaccine is a homogeneous white suspension.

The *Corynebacterium diphtheriae* and *Clostridium tetani* organisms are grown in media according to the method of Mueller and Miller[1,2] and are detoxified by use of formaldehyde. The diphtheria and tetanus toxoids are refined by the Pillemer alcohol fractionation method[3] and are diluted with a solution containing phosphate buffer, glycine, and thimerosal (mercury derivative) as a preservative. The acellular pertussis vaccine component is prepared by growing Phase I *Bordetella pertussis* in Stainer-Scholte defined medium and harvesting the culture fluid.

Purification of the acellular pertussis vaccine component is accomplished by ammonium sulfate fractionation steps and a final sucrose density gradient centrifugation. The acellular pertussis vaccine component is detoxified with formaldehyde and thimerosal (mercury derivative) is added as a preservative.

The Diphtheria Toxoid, Tetanus Toxoid, and Acellular Pertussis Vaccine are combined, diluted in phosphate buffered saline (PBS), and adsorbed to aluminum. The aluminum adjuvant content by assay is ≤ 0.85 mg aluminum per 0.5 mL dose and is present as aluminum hydroxide and aluminum phosphate. The residual free formaldehyde content by assay is ≤ 0.02%. Thimerosal (mercury derivative) is present in a final concentration of 1:10,000. The final product may also contain gelatin and polysorbate 80 which are used in early stages of the process.

Each 0.5 mL dose is formulated to contain 7.5 Lf of diphtheria toxoid and 5.0 Lf of tetanus toxoid (both toxoids induce not less than 2 units of antitoxin per mL in the guinea pig potency test) and 300 hemagglutinating (HA) units of Acellular Pertussis Vaccine. A hemagglutination unit is that amount of material which completely agglutinates chicken red blood cells as measured by the HA assay.[4] The acellular pertussis vaccine component contains approximately 40 ug (but not more than 60 ug) of pertussis antigens per 0.5 mL dose with approximately 86% filamentous hemagglutinin (FHA); approximately 8% lymphocytosis promoting factor (LPF); approximately 4% per dose 69-kilodalton (69kd) outer membrane protein, and approximately 2% type 2 fimbriae (pertussis-specific agglutinogen).

The potency of the pertussis component is evaluated by measurement of ELISA titers in immunized mice against FHA, LPF, and 69kd and fimbriae.

The acellular pertussis vaccine component is produced by Takeda Chemical Industries, Ltd., Osaka, Japan and is combined with diphtheria and tetanus toxoids manufactured by Lederle Laboratories. The bulk vaccine is prepared by Lederle Laboratories. ACEL-IMUNE is filled, labeled, packaged and released by Lederle Laboratories.

CLINICAL PHARMACOLOGY

Simultaneous immunization against diphtheria, tetanus, and pertussis (whooping cough) during infancy and childhood has been a routine practice in the US since the late 1940s. It has played a major role in markedly reducing the incidence of cases and deaths from each of these diseases.

Diphtheria is primarily a localized and generalized intoxication caused by diphtheria toxin, and extracellular protein metabolite of toxinogenic strains of *Corynebacterium diphtheriae*. While the incidence of diphtheria in the US has decreased from over 200,000 cases reported in 1921, before the general use of diphtheria toxoid, to only 15 cases reported from 1980 to 1983,[5] case fatality rate has remained constant at about 5% to 10%.

The highest case fatality rates are in the very young and in the elderly.

Following adequate immunization with diphtheria toxoid it is thought that protection lasts for at least 10 years.[3] Antitoxin levels of at least 0.01 antitoxin units/mL are generally regarded as protective.[4] This significantly reduces both the risk of developing diphtheria and the severity of clinical illness. It does not, however, eliminate carriage of *C diphtheriae* in the pharynx or on the skin.[3]

Tetanus is an intoxication manifested primarily by neuromuscular dysfunction caused by a potent exotoxin elaborated by *Clostridium tetani*. The incidence of tetanus in the US has dropped dramatically with the routine use of tetanus toxoid, remaining relatively constant over the last decade at about 90 cases reported annually. Spores of *C tetani* are ubiquitous, and there is essentially no natural immunity to tetanus toxin.

Thus, universal primary immunization with tetanus toxoid with subsequent maintenance of adequate antitoxin levels, by means of timed boosters, is necessary to protect all age groups.[3] Tetanus toxoid is a highly effective antigen, and a completed primary series generally induces serum antitoxin levels of at least 0.01 antitoxin units, a level which has been reported to be protective.[7] It is thought that protection persists for at least 10 years.[3]

Pertussis (whooping cough) is a highly communicable disease of the respiratory tract that has an attack rate in unimmunized household contacts of over 90%.[3] Since immunization against pertussis (whooping cough) became widespread, the number of reported cases and associated mortality in the US has declined from about 120,000 cases and 1,100 deaths in 1950,[8] to an annual average of about 3,500 cases and 10 fatalities in recent years.[9,10] Precise data do not exist, since bacteriological confirmation of pertussis can be obtained in less than half of the suspected cases. Most reported illness from *B pertussis* occurs in infants and young children; two thirds of reported deaths occur in children less than 1 year old. Older children and adults, in whom classic signs are often absent, may go undiagnosed and serve as reservoirs of disease.[3]

Pertussis disease (whooping cough) is caused by a gram-negative coccobacillus, *B pertussis*. Several antigens which are thought to play a role in protective immunity have been isolated from cultures of *B pertussis*. These include filamentous hemagglutinin (FHA), lymphocytosis promoting factor (LPF), also known as pertussis toxin (PT), a 69-kilodalton (69kd) outer membrane protein, and fimbriae (pertussis-specific agglutinogens).[10,11,12] Another biologically active component, endotoxin, may contribute to reactogenicity of pertussis vaccines.[13] The Takeda acellular pertussis vaccine component used in ACEL-IMUNE contains inactivated LPF, FHA, 69kd outer membrane protein and type 2 fimbriae (pertussis-specific agglutinogen), with minimal endotoxin compared to that in whole-cell pertussis vaccine. The pertussis component induces immunity against pertussis (whooping cough).

Acellular pertussis vaccines have been used in Japan since 1981, mostly in 2-year-old children. Evidence for the efficacy of these vaccines, as a group, is demonstrated by the decline in pertussis disease with their routine use in that country.[14,15] In addition, a review of epidemiological studies of the Japanese acellular pertussis vaccines estimated that these vaccines, as a group, were 88% efficacious in protecting against clinical pertussis on household exposure, with a 95% confidence interval of 79% to 93%.[16] In three Japanese household contact studies which employed retrospective case ascertainment and non-standard case definitions, the vaccine-specific efficacy of the Takeda vaccine ranged between 89% and 94% but confidence intervals were wide, due to the small number of children in each study.[16,17,18,19,20] Although there were differences in study methods, these estimates of efficacy are quite comparable to that for whole-cell pertussis vaccine in the United States.[17]

Efficacy of the DTP vaccine containing the Takeda acellular pertussis vaccine component was examined, in particular, in a non-blinded household contact study, conducted by Lederle Laboratories, that included both retrospective and prospective case evaluation.[21] As a consequence of the immunization schedule in Japan at the time of study, none of the vaccinated contacts were less than 2 years of age while some of the unvaccinated contacts were less than 2 years of age. When analysis of results was limited to vaccinated and unvaccinated household contacts 2 years of age and over, efficacy was estimated to be 79% (95% confidence interval, 60 to 89%) for physician-diagnosed pertussis disease. This included respiratory illnesses that may have been mild per-

tussis. When cases were restricted to disease diagnosed as typical pertussis, omitting mild suspect cases, efficacy was estimated to be 97% (95% confidence interval, 82 to 99%).

When unvaccinated household contacts under 2 years of age are also included in the analysis, efficacy was estimated to be 81% (95% confidence interval, 64 to 90%) against pertussis disease (including mild suspect cases) and 98% (95% confidence interval, 84 to 99%) against typical pertussis. While there is some uncertainty with regard to the absolute magnitude of these estimates, the data as a whole demonstrate the efficacy of the Lederle Pertussis Vaccine.

Immunogenicity of ACEL-IMUNE compared with whole-cell DTP was studied in approximately 1000 US children receiving these vaccines as a fourth or fifth dose at 17 to 24 months or 4 to 6 years of age. Antibody response following ACEL-IMUNE was similar to whole-cell DTP for LPF, 69kd protein, and agglutinins, and higher than DTP for FHA (the DTP used in these comparative studies was manufactured by Lederle Laboratories). All children achieved protective antibody levels to diphtheria and tetanus toxoids. A serologic correlate to protection against pertussis disease has not been established.[22] ACEL-IMUNE was less reactogenic than the whole-cell DTP vaccine (manufactured by Lederle Laboratories) in these studies[23-24-25] with regard to local reactions including less pain/tenderness, erythema, induration and warmth at the injection site. In addition, there was less drowsiness, fretfulness, fever and antipyretic use following ACEL-IMUNE as compared with DTP. The relative frequency of rare events that may be associated with immunization can only be determined in large post-marketing surveillance studies.

INDICATIONS AND USAGE

Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed, ACEL-IMUNE™ is indicated as a fourth and/or fifth dose for children from 17 months of age up to age 7 years (prior to seventh birthday) who have previously been immunized against diphtheria, tetanus, and pertussis with three or four doses of whole-cell DTP vaccine. The administration of ACEL-IMUNE may be considered for children as young as 15 months of age when it is expected that the child will not return at 18 months to receive the fourth dose in this immunization series although studies in this age group have not been completed.

THIS PRODUCT IS NOT RECOMMENDED FOR USE IN CHILDREN BELOW THE AGE OF 15 MONTHS.

Children who have recovered from culture-confirmed pertussis need not receive further doses of a pertussis-containing vaccine.[5] This vaccine is intended for active immunization against diphtheria, tetanus and pertussis, and is not to be used for treatment of actual infection.

If a contraindication to the pertussis vaccine component occurs, Diphtheria and Tetanus Toxoids, Adsorbed for pediatric use (DT) should be substituted for each of the remaining doses.

As with any vaccine, ACEL-IMUNE may not protect 100% of individuals receiving the vaccine.

CONTRAINDICATIONS

HYPERSENSITIVITY TO ANY COMPONENT OF THE VACCINE, INCLUDING THIMEROSAL, A MERCURY DERIVATIVE, IS A CONTRAINDICATION.

IMMUNIZATION SHOULD BE DEFERRED DURING THE COURSE OF ANY FEBRILE ILLNESS OR ACUTE INFECTION. A MINOR AFEBRILE ILLNESS SUCH AS A MILD UPPER RESPIRATORY INFECTION IS NOT USUALLY REASON TO DEFER IMMUNIZATION.[5-26]

DATA ON THE USE OF ACEL-IMUNE IN CHILDREN FOR WHOM WHOLE-CELL PERTUSSIS VACCINE IS CONTRAINDICATED ARE NOT AVAILABLE. UNTIL SUCH DATA ARE AVAILABLE, IT WOULD BE PRUDENT TO CONSIDER THE IMMUNIZATION PRACTICES ADVISORY COMMITTEE (ACIP) AND AMERICAN ACADEMY OF PEDIATRICS (AAP) CONTRAINDICATIONS TO WHOLE-CELL PERTUSSIS VACCINE AS CONTRAINDICATIONS TO ACEL-IMUNE.

IMMUNIZATION WITH ACEL-IMUNE IS CONTRAINDICATED IF THE CHILD HAS EXPERIENCED ANY EVENT FOLLOWING PREVIOUS IMMUNIZATION WITH PERTUSSIS VACCINE (DTP or acellular pertussis-containing vaccine), WHICH IS CONSIDERED BY THE AAP OR ACIP TO BE A CONTRAINDICATION TO FURTHER DOSES OF PERTUSSIS VACCINE.

THE ACIP STATES THAT "IF ANY OF THE FOLLOWING EVENTS OCCUR IN TEMPORAL RELATION TO RECEIPT OF DTP, THE DECISION TO GIVE SUBSEQUENT DOSES OF VACCINE CONTAINING THE PERTUSSIS COMPONENT SHOULD BE CAREFULLY CONSIDERED...

CONTRAINDICATIONS AND PRECAUTIONS TO FURTHER DTP VACCINATION

CONTRAINDICATIONS

AN IMMEDIATE ANAPHYLACTIC REACTION.

ENCEPHALOPATHY OCCURRING WITHIN 7 DAYS FOLLOWING DTP VACCINATION.

PRECAUTIONS

TEMPERATURE OF ≥ 40.5°C (105°F) WITHIN 48 HOURS NOT DUE TO ANOTHER IDENTIFIABLE CAUSE

COLLAPSE OR SHOCK-LIKE STATE (HYPOTONIC-HYPORESPONSIVE EPISODE) WITHIN 48 HOURS.

PERSISTENT, INCONSOLABLE CRYING LASTING ≥ 3 HOURS, OCCURRING WITHIN 48 HOURS

CONVULSIONS WITH OR WITHOUT FEVER OCCURRING WITHIN 3 DAYS

ALTHOUGH THESE EVENTS WERE CONSIDERED ABSOLUTE CONTRAINDICATIONS IN PREVIOUS ACIP RECOMMENDATIONS, THERE MAY BE CIRCUMSTANCES, SUCH AS A HIGH INCIDENCE OF PERTUSSIS, IN WHICH THE POTENTIAL BENEFITS OUTWEIGH POSSIBLE RISKS, PARTICULARLY BECAUSE THESE EVENTS ARE NOT ASSOCIATED WITH PERMANENT SEQUELAE.[5-26]

The occurrence of any type of neurological symptoms or signs, including one or more convulsions (seizures) following administration of ACEL-IMUNE or whole-cell DTP vaccine is generally a contraindication to further use. The presence of any evolving or changing disorder affecting the central nervous system is a contraindication to administration of pertussis vaccine regardless of whether the suspected neurological disorder is associated with occurrence of seizure activity of any type.[5-26]

The ACIP and the AAP recognize certain circumstances in which children with stable central nervous system disorders, including well-controlled seizures or satisfactorily explained single seizures, may receive pertussis vaccine. The ACIP and AAP do not consider a family history of seizures to be a contraindication to pertussis vaccine.[5-26-27]

The decision to administer a pertussis-containing vaccine to such children must be made by the physician on an individual basis, with consideration of all relevant factors, and assessment of potential risks and benefits for that individual. The physician should review the full text of ACIP and AAP guidelines prior to considering vaccination for such children.[5-26-27] The parent or guardian should be advised of the potential increased risk involved.

There are no data on whether the prophylactic use of antipyretics can decrease the risk of febrile convulsions. However, data suggest that acetaminophen will reduce the incidence of postvaccination fever. The ACIP and AAP suggest administering acetaminophen at age-appropriate doses at the time of vaccination and every 4 to 6 hours to children at higher risk for seizures than the general population.[5-26-27]

The clinical judgment of the attending physician should prevail at all times.

WARNINGS

THIS PRODUCT IS NOT RECOMMENDED FOR USE IN CHILDREN BELOW THE AGE OF 15 MONTHS. STUDIES IN CHILDREN 15-17 MONTHS OF AGE HAVE NOT BEEN COMPLETED.

NO DETERMINATION OF EFFICACY IN INFANTS HAS BEEN MADE TO DATE. STUDIES DESIGNED TO EVALUATE EFFICACY IN INFANTS ARE ONGOING BUT ARE NOT YET COMPLETE. IN ONE IMMUNOGENICITY STUDY, INFANTS RECEIVING ACEL-IMUNE EXHIBITED REDUCED RESPONSES TO LPF AND AGGLUTINOGENS, SIMILAR RESPONSES TO 69kd PROTEIN AND HIGHER SEROLOGICAL RESPONSES TO FHA, COMPARED TO THOSE RECEIVING LEDERLE WHOLE-CELL DTP VACCINE.[22] THE ROLE OF SERUM ANTIBODIES TO PERTUSSIS ANTIGENS IN PROTECTION AGAINST PERTUSSIS DISEASE IS UNKNOWN.

THIS PRODUCT IS NOT RECOMMENDED FOR IMMUNIZING PERSONS ON OR AFTER THEIR SEVENTH BIRTHDAY.

DATA ON THE USE OF ACEL-IMUNE IN CHILDREN FOR WHOM WHOLE-CELL PERTUSSIS VACCINE IS CONTRAINDICATED ARE NOT AVAILABLE. UNTIL SUCH DATA ARE AVAILABLE IT WOULD BE PRUDENT TO CONSIDER ACIP AND AAP CONTRAINDICATIONS TO WHOLE-CELL PERTUSSIS VACCINE AS CONTRAINDICATIONS TO ACEL-IMUNE. (See **CONTRAINDICATIONS.**)

ACEL-IMUNE should be given with caution to children with thrombocytopenia or any coagulation disorder that would contraindicate intramuscular injection. (See **DRUG INTERACTIONS.**)

Routine immunization should be deferred during an outbreak of poliomyelitis, providing the patient has not sustained an injury that increases the risk of tetanus and providing an outbreak of diphtheria or pertussis does not occur simultaneously.

PRECAUTIONS

General

1. PREVIOUS IMMUNIZATION HISTORY SHOULD BE ASCERTAINED TO CONFIRM THAT AT LEAST THREE DOSES OF WHOLE-CELL DTP VACCINE HAVE BEEN GIVEN.
2. PRIOR TO ADMINISTRATION OF ANY DOSE OF ACEL-IMUNE, THE PARENT OR GUARDIAN SHOULD BE ASKED ABOUT THE PERSONAL HISTORY, FAMILY HISTORY, AND RECENT HEALTH STATUS. THE PHYSICIAN SHOULD ASCERTAIN PREVIOUS IMMUNIZATION HISTORY, CURRENT HEALTH STATUS AND OCCURRENCE OF ANY SYMPTOMS AND/OR SIGNS OF AN ADVERSE EVENT AFTER PREVIOUS IMMUNIZATIONS IN THE CHILD TO BE IMMUNIZED, IN ORDER TO DETERMINE THE EXISTENCE OF ANY CONTRAINDICATION TO IMMUNIZATION WITH ACEL-IMUNE AND TO ALLOW AN ASSESSMENT OF BENEFITS AND RISKS.
3. BEFORE THE INJECTION OF ANY BIOLOGICAL, THE PHYSICIAN SHOULD TAKE ALL PRECAUTIONS KNOWN FOR THE PREVENTION OF ALLERGIC OR ANY OTHER SIDE REACTIONS. This should include, a review of the patient's history regarding possible sensitivity; the ready availability of epinephrine 1:1000 and other appropriate agents used for control of immediate allergic reactions; and a knowledge of the recent literature pertaining to use of the biological concerned, including the nature of side effects and adverse reactions that may follow its use.
4. Children with impaired immune responsiveness, whether due to the use of immunosuppressive therapy (including irradiation, corticosteroids, antimetabolites, alkylating agents, and cytotoxic agents), a genetic defect, human immunodeficiency virus (HIV) infection, or other causes, may have reduced antibody response to active immunization procedures.[5-26-28] Deferral of administration of vaccine may be considered in individuals receiving immunosuppressive therapy.[5-28] Other groups should receive this vaccine according to the usual recommended schedule.[5-26-28-29] (See **DRUG INTERACTIONS.**)
5. This product is not contraindicated for use in individuals with HIV.
6. *Since this product is a suspension containing an adjuvant, shake vigorously to obtain a uniform suspension prior to withdrawing each dose from the multiple dose vial.*
7. A separate sterile syringe and needle or a sterile disposable unit should be used for each individual patient to prevent transmission of hepatitis or other infectious agents from one person to another. Needles should be disposed of properly and should not be recapped.
8. Special care should be taken to prevent injection into a blood vessel.

NATIONAL CHILDHOOD VACCINE INJURY ACT

This Act requires that the manufacturer and lot number of the vaccine administered be recorded by the health care provider in the vaccine recipient's permanent medical record, along with the date of administration of the vaccine and the name, address, and title of the person administering the vaccine.

The Act further requires the health care provider to report to a health department or to the FDA the occurrence following immunization of any event set forth in the Vaccine Injury Table including: anaphylaxis or anaphylactic shock within 24 hours, encephalopathy or encephalitis within 7 days, shock-collapse or hypotonic-hyporesponsive collapse within 7 days, residual seizure disorder, any acute complication or sequelae (including death) of above events, or any event that would contraindicate further doses of vaccine, according to this ACEL-IMUNE package insert.[30]

The U.S. Department of Health and Human Services has established a new Vaccine Adverse Event Reporting System (VAERS) to accept all reports of suspected adverse events after the administration of any vaccine, including but not limited to the reporting of events required by the National Childhood Vaccine Injury Act of 1986.[5] The VAERS toll-free number for VAERS forms and information is 800-822-7967.

INFORMATION FOR PATIENT

PRIOR TO ADMINISTRATION OF THIS VACCINE, HEALTH CARE PERSONNEL SHOULD INFORM THE PARENT, GUARDIAN, OR OTHER RESPONSIBLE ADULT OF THE RECOMMENDED IMMUNIZATION SCHEDULE FOR PROTECTION AGAINST DIPHTHERIA, TETANUS AND PERTUSSIS AND THE BENEFITS AND RISKS TO THE CHILD RECEIVING A VACCINE CONTAINING AN ACELLULAR PERTUSSIS COMPONENT. GUIDANCE SHOULD BE PROVIDED ON MEASURES TO BE TAKEN SHOULD ADVERSE EVENTS OCCUR, SUCH AS, ANTIPYRETIC MEASURES FOR ELEVATED TEMPERATURES AND THE NEED TO REPORT ADVERSE EVENTS TO THE HEALTH CARE PROVIDER. PARENTS SHOULD BE PROVIDED WITH VACCINE INFORMATION SHEETS (WHEN AVAILABLE FROM THE CENTERS FOR DISEASE CONTROL) AT THE TIME OF EACH VACCINATION, AS STATED IN THE NATIONAL CHILDHOOD VACCINE INJURY ACT.[30]

DRUG INTERACTIONS

Children receiving immunosuppressive therapy may have a reduced response to active immunization procedures.[5-26-28]

As with other intramuscular injections, ACEL-IMUNE should be given with caution to children on anticoagulant therapy.

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY

ACEL-IMUNE has not been evaluated for its carcinogenic, mutagenic potentials or impairment of fertility.

PEDIATRIC USE

This product is not recommended for use in children below the age of 15 months. Studies in children under 15-17 months of age have not been completed.

No determination of efficacy in infants has been made to date. Studies designed to evaluate efficacy in infants are ongoing but are not yet complete. In one immunogenicity study infants receiving ACEL-IMUNE exhibited reduced responses to LPF and agglutinogens, similar responses to 69kd protein and higher serological responses to FHA, compared to those receiving Lederle Laboratories' whole-cell DTP vaccine.[22] The role of serum antibodies to pertussis antigens in protection against pertussis disease is unknown.

The vaccine is not recommended for use as a primary series in children of any age.

For immunization of children 7 years of age and older, Tetanus and Diphtheria Toxoids, Adsorbed for Adult Use (Td) is recommended.[5-26] If a contraindication to the pertussis component exists, Diphtheria and Tetanus Toxoids Adsorbed for pediatric use (DT) should be substituted.

ADVERSE REACTIONS

Adverse reactions associated with ACEL-IMUNE have been evaluated in 911 children receiving this vaccine as the fourth or fifth dose in the DTP series. The percent of children experiencing common symptoms at any time within 72 hours following immunization is summarized below.[23]

Symptom	% of children* reporting symptoms within 72 hours of immunization (n = 911)
Tenderness	26
Erythema (≥ 2 cm)	10
Induration (≥ 2 cm)	7
Injection site temp	17
Fever ≥ 38°C (100.4°F)	19
≥ 39°C (102.2°F)	1.5
Drowsiness	6
Fretfulness	17
Vomiting	2

*Children age groups 17-24 months and 4-6 years of age (fourth and fifth doses) are included.

During a 72-hour period following immunization, the most frequently reported adverse events, excluding those listed above, in decreasing order of frequency were: upper respiratory infection/rhinitis (6%), diarrhea/loose stools (3.5%), rash (1.2%). One child experienced a febrile seizure 78 hours after immunization.[23] A cause and effect relationship between these latter events and vaccination has not been established.

In investigational studies in 2,041 infants administered a total of 5,719 doses of ACEL-IMUNE the combined frequency of common symptoms, at any time within 72 hours following any dose was as follows: erythema ≥ 2 cm, 4%; induration ≥ 2 cm, 1.5%; fever ≥ 38°C (100.4°F), 7%; drowsiness, 12%; fretfulness, 20%; vomiting, 3%. During this period, events judged by the investigators to contraindicate further doses of vaccine occurred in the indicated number of children: persistent or unusual cry (11); fever ≥ 40.5°C (104.9°F) (1); possible seizure (1); hypotonic-hyporesponsive episode (1); lethargy (1); injection site rash (1). One child died suddenly 6 weeks after immunization following apparent recovery from an enteroviral meningitis;[23] however, a causal relationship with ACEL-IMUNE has not been established.

As with other aluminum-containing vaccines,[31] a nodule may occasionally be palpable at the injection site for several weeks. Although not seen in studies with ACEL-IMUNE, sterile abscess formation or subcutaneous atrophy at the injection site may also occur.

As with any vaccine, there is the possibility that broad use of ACEL-IMUNE could reveal adverse reactions not observed in clinical trials. Events have been reported following administration of other vaccines containing diphtheria, tetanus and/or pertussis antigens. These include those listed below.

Urticaria, erythema multiforme or other rash, arthralgias[32] and, more rarely, a severe anaphylactic reaction (eg, urticaria with swelling of the mouth, difficulty breathing, hypotension, or shock) have been reported following administration of preparations containing diphtheria, tetanus, and/or pertussis antigens.

Neurological complications,[33] such as convulsions,[32] encephalopathy[32-34] and various mono- and polyneuropathies,[34-40] including Guillain-Barre syndrome[41-42] have been reported following administration of preparations containing diphtheria, tetanus, and/or pertussis antigens.

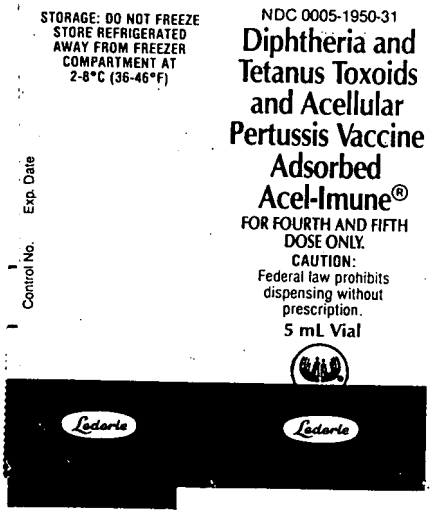
Permanent neurological disability and death have been reported rarely in temporal relation to immunization with vaccines containing pertussis antigens.

11693-91

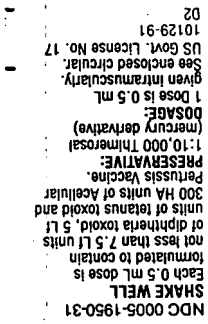
Re: Petition for Extension
of Patent Term for
USPN 4,455,297

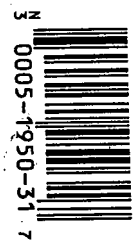
PACKAGE FOR THE

DTP 5 ML. VIAL



3647-1

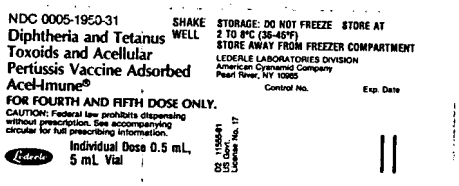




NDC 0005-1950-31
Diphtheria and
Tetanus Toxoids
and Acellular
Pertussis Vaccine
Adsorbed
Acel-Imune®

Re: Petition for Extension
of Patent Term for
USPN 4,455,297

5 ML. VIAL LABEL



100 - 5 mL **1950-31**

DIPHTHERIA and
TETANUS TOXOIDS and
ACELLULAR PERTUSSIS
VACCINE ADSORBED
ACEL-IMUNE®
STORAGE: DO NOT FREEZE.
STORE AT 2 TO 8°C (36-46°F).
STORE AWAY FROM FREEZER COMPARTMENT.
R D1 10362-91

Lederle LEDERLE LABORATORIES DIVISION
American Cyanamid Company, Pearl River, NY 10965

Control No. **Exp. Date**

10362-91* - 4 x 3¼

Re: Petition for Extension
of Patent Term for
USPN 4,455,297

SHIPPING CARTON LABEL

EXHIBIT 2

United States Patent [19]

Syukuda et al.

[11] **4,455,297**

[45] **Jun. 19, 1984**

[54] **METHOD FOR PRODUCING PERTUSSIS TOXOID**

[75] Inventors: **Yukio Syukuda; Hideo Watanabe; Shigeo Matsuyama, all of Hikari, Japan**

[73] Assignee: **Takeda Chemical Industries, Ltd., Osaka, Japan**

[21] Appl. No.: **408,563**

[22] Filed: **Aug. 16, 1982**

Related U.S. Application Data

[63] Continuation of Ser. No. 229,931, Jan. 30, 1981, abandoned.

[30] **Foreign Application Priority Data**

Sep. 12, 1980 [JP] Japan 55-127825

[51] **Int. Cl.**³ **A61K 39/10; C07G 7/00; C12P 21/00**

[52] **U.S. Cl.** ..... **424/92; 424/88; 435/68; 260/112 R**

[58] **Field of Search** ..... 424/88-92; 435/68; 260/112 R

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,135,662	6/1964	Pope et al.	424/92
4,029,765	6/1977	Helting et al.	424/92
4,033,819	7/1977	Helting et al.	424/92
4,075,321	2/1978	Relyveld	424/92
4,242,270	12/1980	Ayme et al.	424/92

Primary Examiner—Blondel Hazel
Attorney, Agent, or Firm—Wegner & Bretschneider

[57] **ABSTRACT**

A pertussis toxoid is produced by removing endotoxin from a culture supernatant of a *Bordetella pertussis* phase I strain or a concentrate thereof and flocculating pertussis exotoxin in the resultant fluid by permitting formaldehyde to act upon the fluid in the substantial absence of basic amino acid. The thus-obtained pertussis toxoid is low in toxicity and has a high immunizing potency.

8 Claims, No Drawings

METHOD FOR PRODUCING PERTUSSIS TOXOID

This application is a continuation of application Ser. No. 229,931 filed Jan. 30, 1981, now abandoned.

This invention relates to a method of producing a pertussis toxoid.

Whooping cough is an infectious disease caused by *Bordetella pertussis* and produces serious effects especially in infants.

Vaccines have heretofore been employed for the prevention of this disease. However, because such vaccines are conventionally prepared from the whole cells of the causative bacterium, they give rise to fever and other serious side effects. It has therefore been an urgent social need to overcome these disadvantages.

Many attempts have been made in which an effective component only is isolated from *Bordetella pertussis* phase I strain and made into a vaccine, but none of the proposed procedures has been found to be satisfactory. Meanwhile, the proposition that the infection by *Bordetella pertussis* lies in the exotoxin released from the said bacteria (M. Pittmann: "Reviews of Infectious Diseases", 1, p. 401–412, 1979) suggested the possibility of protection by means of a pertussis toxoid but there has been no report indicating the success of obtaining a pertussis toxoid.

Against the above technical background, the present inventors have for the first time succeeded in producing a pertussis toxoid by a new method of detoxification.

Thus, the object of this invention is to provide a method of producing a pertussis toxoid which is low in toxicity and yet has a very high immunizing potency.

The said object can be realized by removing endotoxin from a culture supernatant or a concentrate thereof and flocculating pertussis exotoxin in the resultant fluid by permitting formaldehyde to act upon the fluid in the substantial absence of basic amino acid.

In accordance with this invention, there is employed a culture supernatant of a *Bordetella pertussis* phase I strain or a concentrate thereof. The cultivation of the *Bordetella pertussis* phase I strain can be carried out in a manner known per se. Thus, for example, the strain is cultivated in a liquid medium (Cohen-Wheeler medium, Stainer & Scholte medium, etc.) at about 35° to 37° C. for about 5 to 7 days. The supernatant of the resulting culture is collected by filtration or centrifugation. Either this supernatant fluid or a concentrate thereof can be used in the subsequent step of removing its endotoxin. The concentrate can be obtained by salting out which is conventional per se. Thus, for example, 2 to 5 kg of ammonium sulfate is added to 10 l each of the culture supernatant and, after mixing, the precipitate formed is collected by an expedient technique such as filtration or centrifugation. This precipitate is then dissolved in a suitable amount of 0.05 M phosphate buffer supplemented with 1 M sodium chloride, and the supernatant is obtained by centrifugal sedimentation or the like procedure to give a concentrated fluid.

In accordance with this invention, the above-mentioned supernatant or concentrate is treated to remove its endotoxin. This removal of the endotoxin can be accomplished by any of such procedures as sucrose density gradient centrifugation, potassium tartrate density gradient centrifugation, cesium chloride density gradient centrifugation, gel filtration, etc. A particularly advantageous procedure comprises centrifuging the above-mentioned supernatant or concentrate on a sucrose density gradient of about 0 to 60 W/W % at R max. about 62,000 to 122,000 G for about 10 to 24 hours.

The most essential feature of this invention is the step of flocculating pertussis exotoxin in the above obtained pertussis exotoxin fluid by permitting formaldehyde to act upon the fluid in the substantial absence of basic amino acid, whereby the exotoxin is substantially detoxified to yield pertussis toxoid. Thus, the precipitated-purified vaccine containing the thus-detoxified toxoid and the precipitated-purified pertussis-diphtheria-tetanus trivalent vaccine containing the same detoxified toxoid are low in toxicity and yet have very high immunizing potencies. Such effects cannot be achieved with the pertussis toxoid fluid prepared by permitting formaldehyde to act upon the pertussis exotoxin fluid in the substantial presence of basic amino acid, especially L-lysine.

Generally, the conventional bacterial exotoxins such as diphtheria toxin give only loose bindings between formaldehyde and toxin molecules and it was impossible to obtain a stable polymerizate without the aid of an additive substance such as a basic amino acid e.g. L-lysine. As regards pertussis exotoxin, however, it has been found unexpectedly that the formalin detoxification in the absence of such amino acid promotes on the contrary the polymerization of the exotoxin to give a flocculent antigen mass. This promotes the increase of immunity-competent molecule size, potentiates the immunogenecity and, hence, enables the production of a high-potency pertussis toxoid.

The above flocculating treatment is carried out by adding formalin (i.e. 37 W/V % aqueous solution of formaldehyde) or a dilution thereof with water to the pertussis exotoxin fluid in the substantial absence (i.e. less than 10 mM) of basic amino acid such as L-lysine and incubating the mixture until the pertussis exotoxin is substantially detoxified. It is usually advantageous to admix formalin or its dilution with the exotoxin fluid, with no addition of basic amino acid at all, to give a concentration of about 0.1 to 0.6 V/V % in terms of formalin and incubate the mixture, with or without further addition of formalin or its dilution up to a total concentration within the above range, at about 32° to 42° C. for about 3 to 14 days.

By the above treatment, the pertussis exotoxin is flocculated and thereby detoxified to yield a flocculent pertussis toxoid mass-containing suspension. The resultant flocculent toxoid mass in the suspension is dispersed by a suitable technique such as ultrasonication at about 10 to 50 kc to give a toxoid fluid.

In the method of this invention, a dialysis treatment may be interposed between the respective steps. Such dialysis can be carried out in a per se conventional manner.

Exactly in the same manner as the whole cell whooping cough vaccine fluid, the pertussis toxoid fluid thus obtained can be processed into a precipitated-purified pertussis vaccine or a precipitated-purified pertussis-diphtheria-tetanus trivalent vaccine and can be administered to humans.

The following Examples are further illustrative but not limitative of this invention.

The properties of Tohama phase I strain of *Bordetella pertussis* employed in the following Examples are disclosed in e.g. "Infection and Immunity", 6, p. 899–904 (1972). This strain has been maintained at National Institute of Health, Tokyo, Japan (NIHJ), and deposited at

also Institute for Fermentation, Osaka, Japan under the accession number of IFO-14073.

Throughout the present specification as well as in claims, the abbreviations "μg", "mg", "g", "kg", "ml", "l", "°C.", "mM", "M", "r.p.m.", "kc", "R max." "G", "IU" and "Lf" respectively refer to "microgram(s),", "milligram(s)", "gram(s)", "kilogram(s)", "milliliter(s)", "liter(s)", "degree(s) centrigrade", "millimolar concentration", "molar concentration", "revolution(s) per minute", "kilocycle(s)", "Radius maximum", "gravity", "international unit(s)" and "Limit of flocculation".

EXAMPLE 1

Tohama phase I strain of *Bordetella pertussis* was inoculated in a Bordet-Gengou medium prepared from potato, peptone, sodium chloride, agar and bovine blood and incubated at 35° C. for 2 days. Then, the translucent circular colonies were picked up and a colony reactive to the K agglutinating antibody was developed again on a Bordet-Gengou medium for use as a seed culture. A production medium was prepared by autoclaving a Cohen-Wheeler liquid medium (Table 1, hereafter) at 121° C. for 60 minutes and cooling it immediately to about 40° C. This medium was preserved at 37° C.

The seed culture prepared above was added to this production medium to give a terminal population of 200 to 300 million cells/ml, stirred well, inoculated into Roux bottles at the dose of 0.2 l per bottle and immediately cultivated in an incubator at 37° C. The incubation period depended on the cell growth conditions. The maximum cell yield was attained at the fifth day when the hemagglutinating (HA) titer of the culture fluid against chick erythrocytes (as determined by the method described in "Infection and Immunity", 7, p. 922-999 (1978) throughout the present specification) was also at a peak level. Therefore, the fluids were pooled and centrifuged, and 20.2 W/V % of ammonium sulfate was added to the supernatant. After stirring well, the mixture was allowed to stand at 4° C. After 7 days, the supernatant was siphoned off and the sediment was collected and centrifuged at 8,000 r.p.m. for 10 minutes. The supernatant was discarded. To the sediment was added 1/10 of the volume of the fluid pool of 1 M sodium chloride—0.05 M phosphate buffer (pH 8.0), and the mixture was stirred well. The mixture was allowed to stand again at 4° C. for 7 days, after which it was centrifuged again and the supernatant was collected (Extract I). This supernatant was rich in fimbriae, leukycytosis promoting factor (hereafter LPF), histamine sensitizing factor (hereafter HSF) and endotoxin but free from cells. Extract I was reconcentrated, an equal volume of saturated ammonium sulfate (adjusted to pH 8.0 with ammonia) was added thereto and the mixture was allowed to stand at 4° C. for 7 days. This ammonium sulfate fraction was centrifuged at 10,000 r.p.m. for 20 minutes to harvest the sediment and 1/300 of the volume of the fluid pool of 1 M sodium chloride—0.05 M phosphate buffer (pH 8.0) was added thereto. After thorough mixing, the mixture was put in a dialysis tube of semipermeable membrane to remove the ammonium sulfate, using a 1 M solution of sodium chloride (pH 8.0) as the external fluid. The dialyzed concentrate was then subjected to the following sucrose density gradient centrifugation.

A previously sterilized centrifugal rotor (capacity 1700 ml) and seal assembly was driven at a low speed and 1300 ml of 5 W/V % to 30 W/V % sucrose solutions were fed by means of a gradient pump. Then, 100 ml of the above dialyzed concentrate was fed and 300 ml of an overlay fluid (0.5 M sodium chloride solution, pH 8.0) was introduced. The rotor was driven at R max. 89,400 G for 18.5 hours.

After centrifugation, 34 W/V % sucrose solution was introduced at a low speed and the fluid within the rotor was collected in 50 to 100 ml fractions (collection of fractions). This collection was commenced from the low sucrose density side and the high HA-reactive (not less than 20 titers per ml, preferably not less than 500 titers per ml) and endotoxin-lean fractions were harvested. The scarcity of endotoxin was judged by a rabbit pyrogenicity test. Thus, each fraction sample was heated at 100° C. for 3 minutes and diluted to 20 HA titers/ml with physiological saline. This dilution was intravenously administered to rabbits at the dose of 1 ml per kg body weight. The fractions which did not cause fever within 3 hours were selected and pooled as the exotoxin fluid.

The exotoxin fluid was diluted with M/250 phosphate buffered saline (pH 7.0) to a proteinaceous N content of about 50 μg/ml. In this step, gelatin, Tween 80 (polyoxyethylene sorbitan monooleate; Kao-Atlas, Japan) and thimerosal were added to give the concentrations of 0.02 W/V % of gelatin, 0.05 V/V % of Tween 80 and 0.01 W/V % of thimerosal. To this fluid, without the addition of any basic amino acid, was added formalin to a concentration of 0.2 V/V % in an incubator at 39° C. and, after thorough mixing, was allowed to stand in the same incubator. After one day, an additional amount of formalin was added to a concentration of 0.3 V/V % and, after thorough mixing, the mixture was further incubated in the same incubator. After an additional 2 days, formalin was further added to a concentration of 0.4 V/V % and the mixture was stirred well and further incubated in the incubator for a total of 5 days. The resulting flocculated toxoic mass-containing suspension was dialyzed against 0.01 V/V % formalin-physiological saline as the external fluid. This dialysis was carried out by dialyzing the above suspension in a dialysis membrane tube against 12.5 times the volume of the internal fluid of said external fluid in a cold room (4° C.) for 2 days, with the external fluid being constantly agitated. The external fluid was replaced with a fresh one 2 days later and the dialysis was repeated. The dialyzed flocculent toxoid suspension was subjected to various tests applicable to pertussis stock vaccine and, then, used as a stock toxoid fluid. Before the preparation of a final bulk, the flocculent toxoid suspension was ultrasonicated (10 kc, 5 min.) and filtered through a 400 mesh strainer (Japanese Industrial Standard) to give a final pertussis toxoid fluid. As a control, the exotoxin fluid was treated with formalin with addition of 0.05 M L-lysine and subsequently treated as above to obtain a control fluid.

The pertussis toxoid fluid obtained as above and the control fluid were each treated according to the method of Levine (Reo Levine, Joseph L. Stone & Louise Wyman: Factors affecting the efficiency of the aluminum adjuvant in diphtheria and tetanus toxoid. J. Immunology 75, p. 301-307, 1955). Thus, each fluid was diluted with M/250 phosphate buffered saline (pH 7.0) to a protenaceous N content of 20 μg/ml or less, followed by addition of aluminum chloride to a concentration of 0.18 W/V %. The mixture was stirred well and adjusted to pH 7.0 with hydrochloric acid or sodium hydroxide

to give an aluminum-precipitated vaccine of about 0.2 mg in terms of aluminum/ml. The properties of these products are shown in Table 2. After statistical processing, LPF is acceptable when it is not more than the equivalent of 0.5 LPU (Leukocytosis-promoting units as determined by the method described in "Medicine and Biology", 83, p. 117-123)/ml and not acceptable when otherwise. Similarly, HSF is acceptable when it is not more than the equivalent of 0.8 HSU (histamine sensitizing units as determined by the method described in "Journal of Biological Standardization", 7 (1979), p. 21-29)/ml and not acceptable when otherwise. The mouse protecting potency, similarly after statistical processing, is acceptable when it is at least 8 IU (challenged 3 weeks after the immunization)/ml or more and not acceptable when otherwise.

TABLE 2

Method of this invention			Detoxification with the addition of L-lysine					
LPF	HSF	Mouse protecting potency	LPF	HSF	Mouse protecting potency	LPF	HSF	Mouse protecting potency
o	o	8.0	x	x	4.2$^{\Delta}$	o	o	3.0$^{\Delta}$
o	o	14.5	x	x	6.9	o	o	3.0$^{\Delta}$
o	o	12.8	o	x	11.3	o	o	7.0
o	o	10.0	o	x	10.0	o	o	5.0
o	o	12.0	o	x	10.2	o	o	2.2$^{\Delta}$
o	o	15.0	o	x	1.5$^{\Delta}$	o	o	8.4
o	o	13.0	x	x	8.0	o	o	2.0$^{\Delta}$
o	o	18.0	x	o	7.5	o	o	3.0$^{\Delta}$
o	o	11.0	o	x	14.1	o	o	1.8$^{\Delta}$
o	o	12.2				o	o	4.5$^{\Delta}$
o	o	15.2				o	o	4.5$^{\Delta}$
o	o	14.3				o	o	8.0
o	o	18.1				o	o	7.7
o	o	15.5				o	o	5.9
		13.5*1			8.2*1			4.7*1

LPF
o: Not more than the equivalent of 0.5 LPU/ml
x: Other than o (Not acceptable)
HSF
o: Not more than the equivalent of 0.8 HSU/ml
x: Other than o (Not acceptable)
Mouse protecting potency: IU/ml
Δ:Insufficient potency (Not acceptable)
*1: Mean value

As is clear from Table 2, in accordance with the detoxification method of this invention, no rejects were found in regard to any of LPF, HSF and the mouse protecting potency throughout 14 consecutive production batches, the mean potency being 13.5 IU/ml. In contrast, when L-lysine had been added, a 23-batch series of production yielded 4 LPF rejects, 8 HSF rejects and 10 potency rejects, and the overall "acceptables" accounted only for 6/23=26%.

TABLE 1

Soluble starch	225 g
NaCl	375 g
KH_2PO_4	75 g
$MgCl_2.6H_2O$	750 ml (8 W/V % fluid)
$CaCl_2$	75 ml (2 W/V % fluid)
$CuSO_4.5H_2O$	112.5 ml (0.1 W/V % fluid)
Sodium L-glutamate	30 g
Nicotinamide	4.5 g
Casamino acid	1800 g
Cysteine hydrochloride	4.5 g
Tris-buffer	12.5 l

The above components were diluted with distilled water to make 150 l, adjusted to pH 7.0 to 7.2 and sterilized. Then, the following substances were added.

Glutathione (reduced form)	50 ml (1 W/V % fluid)
$FeSO_4.7H_2O$	50 ml (1 W/V % fluid)

EXAMPLE 2

The pertussis toxoid fluid obtained in Example 1, the diphtheria toxoid fluid meeting the Japanese Biological Products Standard and the tetanus toxoid meeting the snme Standard were precipitation-treated as in Example 1 to prepare a precipitated-purified pertussis-diphtheria-tetanus trivalent vaccine. The composition of this vaccine was as follows:

Pertussis toxoid	Proteineous N content; ca. 15 μg/ml
Diphtheria toxoid	ca. 30 Lf/ml
Tetanus toxoid	ca. 5 Lf/ml
Aluminum	ca. 0.2 mg/ml
Thimerosal	0.01 W/V %

The principal properties of this trivalent vaccine are as follows: Hydrogen ion concentration (reciprocal), 7.0; rabbit pyrogenicity (diluted 50-fold with saline and injected intravenously at 1 ml/kg body weight), negative; mouse body weight loss, not more than the equivalent of 10 BWDU (Body weight decrease units as determined by the method described in J. Med. Sci. Biol. 21, 115-135)/ml; mouse leukocytosis promoting activity, not more than the equivalent of 0.5 LPU/ml; mouse histamine sensitizing activity, not more than the equivalent of 0.8 HSU/ml; pertussis toxoid potency, the equivalent of 8 IU/ml; diphtheria toxoid potency, the equivalent of 45 IU/ml; tetanus toxoid potency, the equivalent of 30 IU/ml.

The trivalent vaccine can be administered to humans, for example, by the following schedule:

To infants of 3 to 48 month-age 0.5 ml each of the vaccine is inoculated subcutaneously 3 times with intervals of 2 to 8 weeks. Twelve to eighteen months after the last inoculation, further 0.5 ml of the vaccine is subcutaneously inoculated to each of the infants.

What is claimed is:

1. A method of producing a pertussis toxoid, which comprises removing endotoxin from a culture supernatant of a *Bordetella pertussis* phase I strain or a concentrate thereof, flocculating pertussis exotoxin in the resultant fluid by permitting formaldehyde to act upon the fluid in the substantial absence of basic amino acid and dispersing the flocculent mass in the resulting suspension of ultrasonication.

2. A method of claim 1, wherein the flocculation is performed by admixing formalin or a dilution thereof with the fluid in the substantial absence of basic amino acid and incubating the mixture.

3. A method of claim 2, wherein the incubation is continued until the pertussis exotoxin is substantially detoxified.

4. A method of claim 2, wherein formalin or a dilution thereof is admixed with the fluid, with no addition of basic amino acid, to give a concentration of about 0.1 to 0.6 v/v % in terms of formalin, and the mixture is incubated at about 32° to 42° C. for about 3 to 14 days.

5. A method of claim 1, wherein the removal of endotoxin is accomplished by contrifuging the culture supernatant or concentrate thereof on a sucrose density gradient of about 0 to 60 w/w % at R max. about 62,000 to 122,000 G for about 10 to 24 hours.

6. A method of claim 1, wherein a dialysis treatment is interposed between the respective steps.

7. A method of claim 1, wherein the culture supernatant is concentrated by salting out with use of ammonium sulfate, and endotoxin is removed from the resulting concentrate.

8. A method of claim 1, wherein *Bordetella pertussis* phase I strain is Tohama phase I strain.

* * * * *

EXHIBIT 3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Patent of

Yukio Syukuda et al.

U.S. Patent No.: 4,455,297

Issued: June 19, 1984

Serial No.: 408,563

Filed: August 16, 1982

For: METHOD FOR PRODUCING PERTUSSIS TOXOID

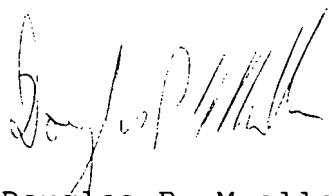
REQUEST FOR CERTIFICATE OF CORRECTION UNDER RULE 322

Honorable Commissioner of
Patents and Trademarks
Washington, DC 20231

Sir:

It is respectfully requested that a Certificate of Correction be issued in order to correct the error specified on the attached copy of the Certificate of Correction Form (PTO-1050) which has been completed according to the Notice in 862 O.G. 2. No fee is included, as this correction was made in the Amendment filed October 13, 1983.

Respectfully submitted,



Douglas P. Mueller
Reg. No. 30,300

WEGNER, CANTOR, MUELLER & PLAYER
P. O. Box 18218
Washington, DC 20036-8218
(202) 887-0400

Attorney Docket No.: P8700-18439A
DATE: February 7, 1992
DPM:ldc/2.53

Staple Here Only !

PRINTER'S TRIM LINE

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 4,455,297

DATED : June 19, 1984

INVENTOR(S) : Yukio Syukuda et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 7, line 8 (last line of claim 1), change "of" to --by--.

MAILING ADDRESS OF SENDER:

WEGNER, CANTOR, MUELLER & PLAYER
P. O. BOX 18218
WASHINGTON, DC 20036-8218

PATENT NO. 4,455,297

No. of add'l. copies @ 30¢ per page

FORM PTO 1050 (REV. 3-82)

RECEIVED

JAN 22 1992

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D. C. 20231

FROHWERK, CANTOR, MUELLER & PLAVEC

WEGNER & BRETSCHNEIDER
P. O. BOX 19542
WASHINGTON, DC 20036

DATE MAILED
1/15/92

97171

MAINTENANCE FEE STATEMENT

The data shown below is from the records of the Patent and Trademark Office. If the maintenance fees and any necessary surcharges have been timely paid for the patents listed below, the notation "PAID" will appear in column 10, "status" below.

If a maintenance fee payment is defective, the reason is indicated by code in column 10, "status" below. An explanation of the codes appears on the reverse of the Maintenance Fee Statement. TIMELY CORRECTION IS REQUIRED IN ORDER TO AVOID EXPIRATION OF THE PATENT. NOTE 37 CFR 1.377. THE PAYMENT(S) WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION. IF PAYMENT OR CORRECTION IS SUBMITTED DURING THE GRACE PERIOD, A SURCHARGE IS ALSO REQUIRED. NOTE 37 CFR 1.20(k) and (l).

If the statement of small entity status is defective the reason is indicated below in column 10 for the related patent number. THE STATEMENT OF SMALL ENTITY STATUS WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION.

ITM NBR	PATENT NUMBER	FEE CDE	FEE AMOUNT	SUR CHARGE	SERIAL NUMBER	PATENT DATE	FILE DATE	PAY YR	SML ENT	S
1	4,455,297	171	495	----	06/408,563	06/19/84	08/16/82	08	NO	P

If the "status" column for a patent number listed above does not indicate "PAID" a code or an asterisk (*) will appear in the "status" column. Where an asterisk (*) appears, the codes are set out below by the related item number. An explanation of the codes indicated in the "status" column and as set out below by the related item number appears on the reverse of the maintenance fee statement.

ITM NBR | STATUS CODE

NUMBERS

NONE

DIRECT THE RESPONSE TOGETHER WITH ANY QUESTIONS ABOUT THIS NOTICE TO:
COMMISSIONER OF PATENTS AND TRADEMARKS, BOX M. FEE, W/

6

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of: **BOX M. FEE**

YUKIO SYUKUDA ET AL

Serial No.	:	408,563
Filed	:	August 16, 1982
U.S. Patent No.	:	4,455,297
Issued	:	June 19, 1984
Title	:	METHOD FOR PRODUCING PERTUSSIS TOXOID

PAYMENT OF SEVEN AND A-HALF YEAR MAINTENANCE FEE

Honorable Commissioner of
Patents and Trademarks
Washington, DC 20231

Sir:

Attached hereto is a check in the amount of $495.00 in payment of the maintenance fee due within seven years and six months after the original grant. This payment is calculated in accordance with 37 CFR 1.20(e), as the application on which this patent issued was filed before August 27, 1982.

Should this check become detached or any fee adjustment be necessary, kindly credit or debit our Deposit Account No. 23-0783 as necessary.

Please forward the maintenance fee receipt to the undersigned at the fee address noted at the bottom of this order for payment.

Respectfully submitted,

Herbert I. Cantor
Reg. No. 24,392

WEGNER, CANTOR, MUELLER & PLAYER
P. O. Box 18218
Washington, DC 20036-8218
(202) 887-0400
Attorney Docket No.: P-8700-18439A
Date: December 12, 1991
HIC:tm\mf12

UNITED STATES PARTMENT OF COMMERCE
Patent and Trademark Office
Address COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D. C. 20231

WEGNER & BRETSCHNEIDER
P. O. BOX 19542
WASHINGTON, DC 20036

DATE MAILED
12/21/87

034428

MAINTENANCE FEE STATEMENT

The data shown below is from the records of the Patent and Trademark Office. If the maintenance fees and any necessary surcharges have been timely paid for the patents listed below, the notation "PAID" will appear in column 10, "status" below.

If a maintenance fee payment is defective, the reason is indicated by code in column 10, "status" below. An explanation of the codes appears on the reverse of the Maintenance Fee Statement. TIMELY CORRECTION IS REQUIRED IN ORDER TO AVOID EXPIRATION OF THE PATENT. NOTE 37 CFR 1.377. THE PAYMENT(S) WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION. IF PAYMENT OR CORRECTION IS SUBMITTED DURING THE GRACE PERIOD, A SURCHARGE IS ALSO REQUIRED. NOTE 37 CFR 1.20(k) and (l).

If the statement of small entity status is defective the reason is indicated below in column 10 for the related patent number. THE STATEMENT OF SMALL ENTITY STATUS WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION.

ITM NBR	PATENT NUMBER	FEE CDE	FEE AMOUNT	SUR CHARGE	SERIAL NUMBER	PATENT DATE	FILE DATE	PAY YR	SML ENT	STA
1	4,455,297	173	450	----	06/408,563	06/19/84	08/16/82	04	NO	PAI

If the "status" column for a patent number listed above does not indicate "PAID" a code or an asterisk (*) will appear in the "status" column. Where an asterisk (*) appears, the codes are set out below by the related item number. An explanation of the codes indicated in the "status" column and as set out below by the related item number appears on the reverse of the maintenance fee statement.

ITM NBR	ATTY DKT NUMBER
1	HCW 18439A

DIRECT T

QUESTIONS ABOUT THIS NOTICE TO

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

Yukio SYUKUDA et al.

Serial No. 408,563

Filed: October 13, 1983

U.S. Patent No. 4,455,297

Issue Date: June 19, 1984

PAYMENT OF THREE AND A HALF-YEAR MAINTENANCE FEE

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

Attached hereto is a check for $450.00 in payment of the maintenance fee due within three years and six months after the original grant. This payment is calculated in accordance with 37 CFR 1.20(h), as the application on which this patent issued was filed on or after August 27, 1982.

Should this check become detached or any fee adjustment be necessary, kindly credit or debit our deposit account No. 23-0783 as necessary.

Early acknowledgement of this payment is courteously solicited.

Respectfully submitted,

Douglas P. Mueller
Reg. No. 30,300

WEGNER & BRETSCHNEIDER
P.O. Box 18218
Washington, D.C. 20036-8218
(202) 887-0400
Atty. Docket No. HCW-18439-A
Date: December 10, 1987
DPM:mwr/lgl.c